

Effect of Concentration and Degree of Saturation of Topical Fluocinonide Formulations on *In Vitro* Membrane Transport and *In Vivo* Availability on Human Skin

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Purpose. The thermodynamic activity of drugs in topical vehicles is considered to significantly influence topical delivery. *In vitro* diffusion across a synthetic membrane was shown to be correlated to the degree of saturation of the drug in the applied vehicle and therefore offers a potential for increased topical drug delivery. Fluocinonide a topical corticosteroid, was chosen as a model compound to investigate *in vitro* and *in vivo* availability from formulations with different degrees of saturation.

Methods. Sub-, as well as, supersaturated drug solutions were prepared using PVP as an antinucleant agent. *In vitro* membrane diffusion experiments across silicone membrane and *in vivo* pharmacodynamic activity assessments, using the human skin blanching assay, were carried out.

Results. Over the concentration range studied, the *in vitro* membrane transport of fluocinonide was proportional to the degree of saturation of the respective formulations. The *in vivo* pharmacodynamic response in the human skin blanching assay was related to the concentration of the drug in the vehicle irrespective of the degree of saturation.

Conclusions. From the membrane permeation experiment it can be concluded, that the drug flux might be increased supra-proportionally with increasing donor concentration, drug (super-)saturation (proportional), beyond what would be anticipated based on ideal donor concentration and partition coefficient considerations only. These findings could not be confirmed in the *in vivo* investigation, probably due to additional vehicle effects (e.g., enhancement, irritation, drug binding) which have to be expected and could have altered the integrity of the stratum corneum and therewith topical bioavailability of the drug.

KEY WORDS: topical availability; supersaturation; silicone membrane; fluocinonide, *in vivo/in vitro* correlation; human skin blanching assay; percutaneous penetration.

INTRODUCTION

The stratum corneum, which is the outermost layer of mammalian skin, typically provides the major barrier to transdermal drug absorption. Numerous strategies have been developed to overcome the skin barrier function in order to deliver

more drug to target sites in the skin or to increase drug permeation through the skin. The diverse techniques of iontophoresis, sonophoresis, electroporation, the use of novel vehicle systems (1) as well as the incorporation of permeation enhancers (2,3) into the vehicle have all been successful in this regard.

To this end, significantly less attention has been paid to the consequences of thermodynamic activity of a solute in a vehicle on membrane transport. Based on the early work of Higuchi (4), Coldman *et al.* (5) were the first to demonstrate that volatile solvent systems could be used to generate states with increased thermodynamic activity (supersaturation) that lead to an increase in percutaneous absorption when compared to that possible from saturated solutions. The correlation between the degree of saturation (subsaturated through saturated systems) and membrane transport is well documented and evidence that supersaturated states may increase transport beyond the limiting value achieved with saturated systems are also available. It is likely that changes in vehicle composition following application to the skin, which increase the activity of the solute in the residual vehicle phase, occurs in many commercial formulations and is possibly necessary for adequate, although generally low, percutaneous absorption and efficacy.

Based on observations made by Yalkowsky *et al.* (6), Davis and Hadgraft (7) recently initiated a systematic investigation of the effect of supersaturation on membrane transport using mixed cosolvent systems. They demonstrated the flux of hydrocortisone acetate from vehicles with a constant drug concentration across silicone membranes increased when the degree of saturation was increased by variation of the cosolvent composition of the vehicle. The use of these mixed cosolvent systems in combination with antinucleant or anticrystal growth agents proved to be an elegant approach to the practical use of supersaturation in enhanced topical drug delivery (8,9).

Due to the fact, that most conventional topical dermatological formulations contain high concentrations of the active agent compared with the fractional amount absorbed, the mixed cosolvent system approach offers a further advantage. To achieve an equivalent bioavailability, the amount of active agent in these formulations may be decreased by simultaneously increasing the solute activity and hence resulting in a lower drug burden and fewer adverse effects. In theory, supersaturation appears to offer a further advantage over "traditional" enhancers in which the enhancement is specific to the compound of interest, there is no breakdown of the barrier function and the absorption of other compounds (e.g., excipients) is not enhanced.

Using this as the background we studied the transport of fluocinonide, a topical corticosteroid, in a mixed cosolvent system (propylene glycol, glycerol, ethanol and water) through a silicone membrane. The *in vitro* data is compared with the findings of an *in vivo* pharmacodynamic response study.

MATERIALS AND METHODS

Materials

Fluocinonide (Syntex; pharmaceutical grade) was obtained from Protochemie Ltd., Mitlödi, Switzerland. Glycerol 98%, polyethylene glycol 400, propylene glycol (Hänseler Ltd., Herisau, Switzerland), polyvinylpyrrolidone K 25 (Fluka Ltd., Buchs, Switzerland), ethanol 96% and purified water (Institute

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of Hospital-Pharmacy, Basel, Switzerland) were all of pharmaceutical grade. Sil-Tec™ silicone-sheeting (8 × 4 × 0.005 inch) was obtained from Technical Products Inc. of Georgia, USA, and isopropyl myristate was purchased from Fluka Ltd., Buchs, Switzerland. Topsy™ solution (Protochemie Ltd., Mitlödi, Switzerland), a brand product containing 0.5 mg/ml fluocinonide and propylene glycol, was used as a reference formulation.

Chromatographic Procedure

Fluocinonide concentrations were assayed by HPLC using a Waters 2690 separations module Alliance™ equipped with a Waters 996 photodiode array detector which were controlled by Millennium™ 2010 software version 2.21 (all Waters Corp., Milford, Massachusetts). The mobile phase consisted of 50% acetonitrile and 50% water (HPLC grade, LiChrosolv™; Merck Ltd., Darmstadt, Germany).

For solubility and stability studies, a LiChrospher 100 RP-18 (5 μm) column (125 × 4 mm; Merck Ltd., Darmstadt, Germany) was used and the flow rate was set at 0.9 ml/min. Standard curves were obtained by injecting 10 μl-aliquots of fluocinonide solutions dissolved in mobile phase. UV detection was carried out at 238 nm with measurement of peak areas. The assay was linear in the working range of 0.2 to 100 μg/ml and the correlation coefficient for the regression of the calibration curve was 0.999.

For membrane permeation studies, a Symmetry C18 5μm column (150 × 2.1 mm; Waters Corp., Milford, Massachusetts) was used and the flow rate was set at 0.3 ml/min. As 10 μl-aliquots of the receptor phase were directly injected onto the HPLC column, calibration standards were dissolved in the same solvent mixture, which consisted of ethanol:polyethylene glycol 400 (1:1). UV detection was done at 238 nm and the area versus concentration curves were determined to be linear from 0.05 to 10 μg/ml with a correlation coefficient of 0.999.

Preparation of Solutions with Different Degrees of Saturation

Solutions with different degrees of saturation (DS = concentration / saturation solubility) were prepared on a volumetric basis using the methods described by Davis *et al.* (7) and Megrab *et al.* (9). The corticosteroid was dissolved in an ethanol:propylene glycol mixture (approximately 1:1), designated as cosolvent system b, and diluted with a water-glycerol mixture (approx. 1:1), the cosolvent system a. Consequently all the resulting mixtures consisted of a volatile part of roughly 50% (water and ethanol) and a 50% fraction of organic cosolvents (glycerol and propylene glycol), where the latter advantageously hinders evaporation of the volatile constituents. Systems with different degrees of saturation were prepared by two different methods. The one method involved dissolving fluocinonide in cosolvent system b (500 μg/ml) followed by dilution with different ratios of cosolvent system a (see series 2 in Table 1, and Fig. 1). The resulting mixtures showed increasing degrees of saturation together with a decreasing concentration of drug. On the other hand solutions with different degrees of saturation were produced by generating a solution of fluocinonide in system b (833.3 μg/ml) which was further mixed with different ratios of cosolvents b and a to produce solutions with a constant concentration of fluocinonide (250 μg/ml) and

Table 1. Design of Fluocinonide Topical Solutions with Different Degrees of Saturation (DS) (All the Solutions Contained Polyvinylpyrrolidone K 25 in a Concentration of 8%), and Steady State Flux and Permeability Coefficients Calculated from the In Vitro Permeation Data Across Silicone Membrane

Series	Solution	Concentration		Flux ^a	Permeability ^b
		(μg/ml)	DS		
1	A	250	3.80	5.50 ± 0.51	22.01 ± 2.04
	B	250	1.59	4.07 ± 0.20	16.26 ± 0.78
	C	250	0.82	2.18 ± 0.16	8.72 ± 0.66
	D	250	0.17	1.66 ± 0.15	6.62 ± 0.58
2	Topsy™	500	0.17	3.88 ± 1.0	7.75 ± 2.05
	E	100	3.76	2.78 ± 0.18	27.79 ± 1.82
	F	150	2.28	2.98 ± 0.37	19.85 ± 2.48
	G	200	1.27	2.81 ± 0.26	14.04 ± 1.29
	C	250	0.82	2.18 ± 0.16	8.72 ± 0.66
	Topsy™	500	0.17	3.88 ± 1.0	7.75 ± 2.05

^a Flux in 10⁻⁵ μg/cm² · sec.

^b Permeability in 10⁻⁸ cm/sec.

different degrees of saturation (see series 1 in Table 1, and Fig. 1).

Determination of Saturation Solubilities

The saturation solubility of fluocinonide was determined for different compositions of a cosolvent system which was formulated with four components (ethanol, propylene glycol, glycerol, and water). Two initial systems consisted of a) water:glycerol (approximately 1:1) and b) ethanol:propylene glycol (approx. 1:1). To produce the initial systems, 50 ml of one of the volatile phases (water or ethanol) was dispensed in a volumetric flask, and the corresponding organic cosolvent phase (glycerol or propylene glycol, respectively) was added to produce 100 ml. The final cosolvent solutions of different proportions were made up by transferring an aliquot of system

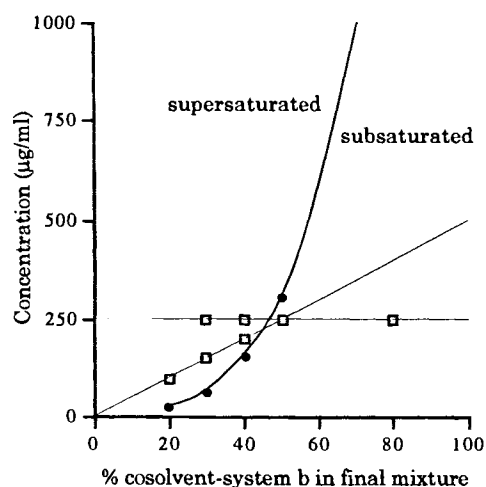


Fig. 1. Design of fluocinonide topical solution systems (open squares) with different degrees of saturation. Solutions on the left side of the saturation solubility curve of fluocinonide in cosolvent mixtures containing 8% polyvinylpyrrolidone K 25 (filled circles) were supersaturated, and those on the right side were subsaturated, respectively.

b into a volumetric flask and adding of solvent a to a final volume of 10 ml.

The saturation solubility of fluocinonide was determined by each of the two methods for comparative purpose: 1) excess drug was added to different cosolvent mixtures as described above, and 2) excess amounts were added to cosolvent system b, in which the solute had a higher solubility compared with system a, and were equilibrated for 3–5 hours. The resultant saturated solutions were then diluted with different volumes of cosolvent system a (a poorer solvent for the drug) which instantaneously resulted in a supersaturated solution. These thermodynamically unstable solutions then spontaneously returned to a saturated state within approx. 6–10 hours by precipitation of the drug. The mixtures were placed in plastic tubes and equilibrated in a shaker incubator at 32°C for 48 hours. After filtration through a Millex-GS™ filter (pore size 0.22 µm; Millipore, Molsheim, France) and appropriate dilution with mobile phase, the solutions were assayed by HPLC. To determine the saturation solubility in Topsy™ solution, excess fluocinonide was added to the brand product, equilibrated, centrifuged and assayed.

Stabilization of Supersaturated Solutions

Due to their inherently high instability, supersaturated systems tend to precipitate spontaneously (10) and therefore have to be stabilized (e.g., by addition of an antinucleant polymer (11–13)). Different anti-nucleating agents were screened for applicability in this system according to the method of Davis and Gordon (14) by preparing supersaturated steroid solutions, adding polymers to each sample, allowing the samples to stand and noting the transparency/turbidity of the solution at several time-points. Since polyvinylpyrrolidones (PVP) were found to be the most promising group of polymers (data not shown), different types and concentrations of agents from this family were studied in greater scrutiny with regard to their ability to inhibit formation and growth of crystals. Supersaturated solutions with different types of PVP's were placed into glass vessels containing an inert, porous, nucleation enhancing membrane (Supor™ 450, Gelman Sciences, Ann Arbor, Michigan, USA), which were kept at 32°C and stirred continuously. Samples were drawn, filtered (see above), diluted and assayed by HPLC to determine exact solution concentrations. The nucleation surface and the stirring would greatly enhance precipitation of the drug and would therefore clearly indicate the level of antinucleation afforded by the polymer in each case.

In Vitro Membrane Permeation Studies

Franz diffusion cells (15) with a diffusional surface area of 1.77 cm² and a receptor volume of 12.3 to 12.4 ml were used to conduct the diffusion experiments (Crown Glass Co. Inc., Somerville, New Jersey). The Sil-Tec™ membrane was cut to appropriate size and soaked in isopropyl myristate for two days, wiped with tissue to remove surface liquid and mounted in the cells. A mixture of ethanol and polyethylene glycol (1:1) was used as receptor phase and was degassed by ultrasonication prior to use. After cell equilibration at 32°C, 1.0 ml of drug formulation maintained at the same temperature was applied to the surface of each membrane and then the donor compartments were covered by Parafilm™ (American National Can, Neenah,

Wisconsin). The donor phases were replaced after 8, 16, and 24 hours, to avoid depletion of vehicle, e.g., by crystallisation of the corticosteroid. At designated intervals, samples of 350 µl were drawn from the receiver compartment and the volumes were replaced by thermostated receptor phase. The samples were directly injected onto the HPLC column. Diffusion experiments were carried out for solutions A through G (n = 5) (Table 1) and for Topsy™ solution (n = 4). The silicone membrane permeation data were plotted for the cumulative amount of drug in the receptor compartment as a function of time. The flux values in the steady state J (µg · cm⁻² · sec⁻¹) and the apparent permeability coefficient P (cm · sec⁻¹) were calculated according to Fick's First Law of diffusion:

$$J = \frac{1}{A} \left(\frac{dM}{dt} \right) = P \cdot (c_D - c_R) \quad (1a)$$

In this equation, A is the surface area of the membrane (1.77 cm²) and $(c_D - c_R)$ is the concentration difference across the membrane. Since the concentration of steroid in the receiver compartment was less than 2% compared to that of the donor compartment in all runs and the solubility for fluocinonide was comparatively high (16.7 mg/ml at 32°C), $(c_D - c_R)$ was approximated by the donor concentration (c_D).

Human Skin Blanching Assay

The human skin blanching assay was used to determine the topical availability of the different supersaturated solutions by measuring a pharmacodynamic response of the corticosteroid. The previously described methodology (16) was modified to comply with the aims of the present investigation. A clinical trial was carried out for each of the two series of fluocinonide topical solutions (see Table 1). Ethical approval was obtained from the Rhodes University Ethical Standards Committee in compliance with the 1964 Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained from each subject.

Eight adhesive labels, from which two 7 × 7 mm squares had been punched, were applied to the flexor aspect of both forearms to demarcate a total of 16 application sites (15 occupied) per arm of each of the 12 volunteers. Each formulation, prepared half an hour in advance, was applied to three of the demarcated sites in a random manner according to a double-blinded application regimen. Three microlitres of each formulation were applied by micropipettes, spread evenly over the application sites using a glass rod, and were occluded with Blenderm™ surgical tape (3M, St. Paul, Minnesota) to prevent evaporation of volatile components. After a contact time of six hours, the occlusive dressing and adhesive labels were removed and each application site was separately washed using cotton-tipped buds and distilled water and dried carefully.

The percentages of total possible blanching scores, obtained by visual response assessment, were plotted against time in hours after formulation application to further calculate the 0- to 32-hour area under the blanching (AUBC) curve by employing the trapezoidal rule. The AUBC values obtained from the application sites of both arms were averaged to get one value for each of the twelve volunteers, and Locke's method (17) was used to calculate 90%-confidence intervals for the AUBC ratios of the different formulations.

RESULTS AND DISCUSSION

Preparation of Supersaturated Solutions

In order to design topical supersaturated solution systems of fluocinonide, saturation solubility was determined for different mixtures of the two cosolvent systems ($n = 2-6$) at 32°C (the skin surface temperature on human forearm (18)). The solubility curve is depicted in Fig. 2 (dotted line). The solubility of fluocinonide increased from approximately $0.08 \mu\text{g/ml}$ in cosolvent system a (water/glycerol) to $2.6 \pm 0.1 \text{ mg/ml}$ in cosolvent system b (ethanol/propylene glycol).

Systems with different degrees of saturation (subsaturated, saturated and supersaturated) were produced as described above. Table 1 gives the compositions of the different solutions tested, and Fig. 1 visualizes the sub- or supersaturation design of the respective solutions which were matched into two series.

In a prescreening experiment, PVP's showed a superior inhibitory effect over hydroxypropyl methylcellulose and carboxymethylcellulose sodium on the crystallization of fluocinonide from supersaturated solutions. A further investigation of four different types of polyvinylpyrrolidones, which were PVP K 12PF, K 17PF, K 25, and K29/30, added in a concentration of 5% (w/v) to solution E (see Table 1), demonstrated that type K 25 had the highest capacity to inhibit crystal formation (Fig. 3).

Subsequently the concentration of polyvinylpyrrolidone K 25, added to all the solutions (sub- as well as supersaturated) was increased to 8%. This resulted in acceptable viscosity (with respect to the convenience of production and application of the solutions to the skin) as well as adequate inhibition of crystallization of drug in the different supersaturated solutions. Figure 4 depicts the stability of those three formulations, which were inherently susceptible to crystallisation, after addition of 8% of polyvinylpyrrolidone K 25 as antinucleant polymer, and under worst case conditions, which were simulated by the presence of a porous membrane and extensive stirring. With the exception of solution A, where a retarded crystallisation still

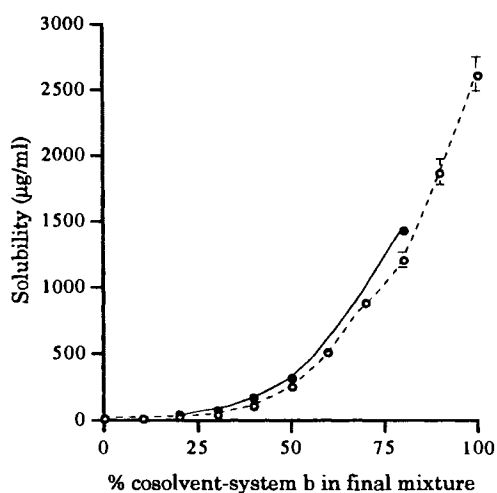


Fig. 2. Saturation solubility of fluocinonide in different mixtures of cosolvent system a (water/glycerol) with cosolvent system b (ethanol/propylene glycol) (open circles); and in the same mixtures containing 8% (w/v) polyvinylpyrrolidone K 25 as antinucleant agent (filled circles). Error bars represent \pm standard deviations.

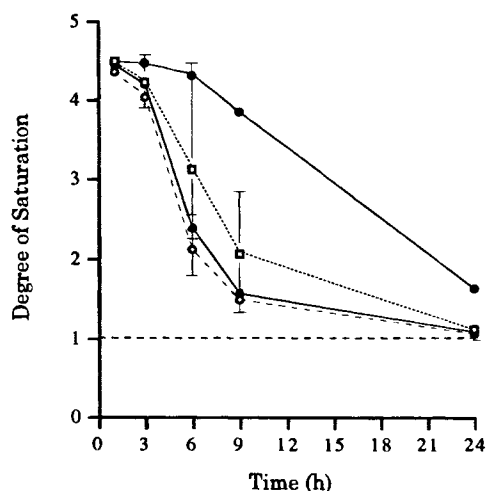


Fig. 3. Stability ($n = 2$) of supersaturated solution E containing 5% (w/v) of different types of polyvinylpyrrolidone (saturation solubility was determined using PVP K12): PVP K 12PF (open squares), PVP K 17PF (open rhombus), PVP K 25 (filled circle), and PVP K 29/30 (open circle). Error bars represent \pm standard deviations.

occurred, all the employed supersaturated solutions had a sufficient stability for the intended utilization.

Since an 8% addition of polyvinylpyrrolidone to the cosolvent mixtures was expected to influence the saturation solubility of fluocinonide, the solubility determination, as done before, was repeated once again at this stage of the work. An enhanced solubility was detected, mainly in the range where the fraction of cosolvent system a was predominant and the solubility was low. The final curve is depicted in Fig. 2 (continuous line) and Fig. 1, and the degrees of saturation as given in Table 1 were calculated from these data.

In Vitro Membrane Permeation Studies

Figure 5 shows the permeation profiles of fluocinonide from different vehicles across the silicone membrane. The

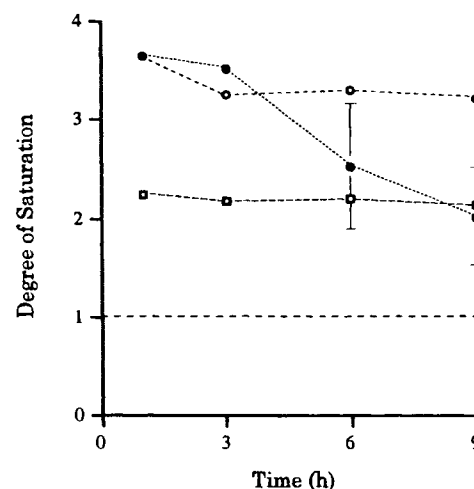


Fig. 4. Stability ($n = 2$) of supersaturated solutions containing 8% (w/v) polyvinylpyrrolidone K 25 as an antinucleant agent: solution A (filled circles), solution E (open circles), and solution F (open squares). Error bars represent \pm standard deviations.

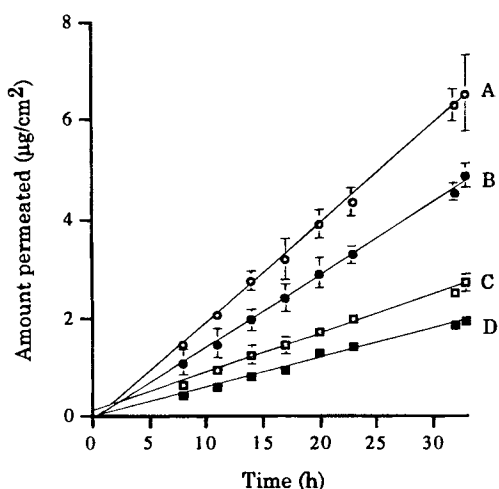


Fig. 5. Amount of fluocinonide permeated across silicone membrane as a function of time. Solutions of series 1: A (open circles), B (filled circles), C (open squares), and D (filled squares). The concentrations were the same (250 µg/ml) for all solutions, but the degree of saturation continually decreased from A, B (supersaturated) to C (almost saturated) and D (subsaturated). Error bars represent \pm standard deviations.

experimental steady-state fluxes and the corresponding permeability coefficients are given in Table 1. These data are analyzed below with respect to the effect of the degree of saturation of the drug solution on membrane permeation. The flux, J , through the membrane is given by Eq. 1b when sink conditions prevail in the receiver compartment and the donor solution shows an ideal behaviour.

$$J = P \cdot c_D \quad (1b)$$

If the system deviates from ideality, the concentration must be replaced by the activity, α :

$$J = P \cdot \alpha_D \quad (2)$$

α is related to c as follows (19):

$$\alpha_D = \gamma_D \cdot c_D \quad (3)$$

where γ is the activity coefficient. The permeability coefficient can be expressed as:

$$P = \frac{D \cdot PC}{h} \quad (4)$$

where, D is diffusion coefficient of the drug in the membrane, h is the thickness of the membrane and PC is drug partition coefficient between the membrane and the donor vehicle. The partition coefficient may be approximated according to Eq 5.

$$PC = \frac{\alpha_{sat,M}}{\alpha_{sat,D}} \approx \frac{c_{sat,M}}{c_{sat,D}} \quad (5)$$

where, the index "sat" indicates saturation solubility, the index "M" indicates membrane and the index "D" indicates donor solution. The use of solubility ratios was shown to provide a good approximation of partition coefficient for a series of steroid hormones, which were similar in structure to the present drug, and for ethanol-water vehicles (20). Apparent permeability coefficients deduced by dividing experimental drug fluxes by

donor concentrations can be expressed using Eqs. 2, 4, and 5 as follows:

$$P_{app} = \frac{J}{c_D} = \frac{P \cdot \alpha_D}{c_D} = \frac{D}{h} \cdot \frac{c_{sat,M}}{c_{sat,D}} \cdot \frac{\alpha_D}{c_D} \quad (6)$$

Furthermore, the degree of saturation, DS , of the donor vehicle may be introduced:

$$DS = \frac{c_D}{c_{sat,D}} \quad (7)$$

By combining Eqs. 3, 6, and 7 the following expression for the apparent permeability coefficient is obtained.

$$P_{app} = \frac{D \cdot c_{sat,M} \cdot \gamma_D}{h \cdot c_D} \cdot DS \quad (8)$$

In Fig. 6, the experimental permeability coefficients are plotted against the degree of saturation. A linear relationship ($r = 0.991$) is obtained for all studied vehicles and all drug concentrations with the exception of vehicle A. The reason for the deviation of vehicle A is considered to be due to the slight crystallization which occurred and lead to a decrease of the respective drug concentration. By applying equation 8 to the data of Fig. 6 it may be concluded that the proportionality term of the equation, which corresponds to the slope of the regression line of the figure, is a constant. D , $c_{sat,M}$ and h depend on the properties of the drug and of the membrane and are considered to be independent of the vehicle used and drug concentration, provided that no interaction between the vehicle and the membrane takes place, which is a reasonable assumption for the silicone membrane. Thus, the ratio γ_D/c_D appears to be constant which means that the activity coefficient is proportional to the donor concentration. The finite y-axis intercept of Fig. 6 can not be interpreted with certainty, it may, however, reflect a rough estimate of permeability in the ideal situation of infinite dilution.

These results suggest the studied drug formulations have a non-ideal behaviour and the activity coefficient in the tested concentration range tested increases approximately linearly

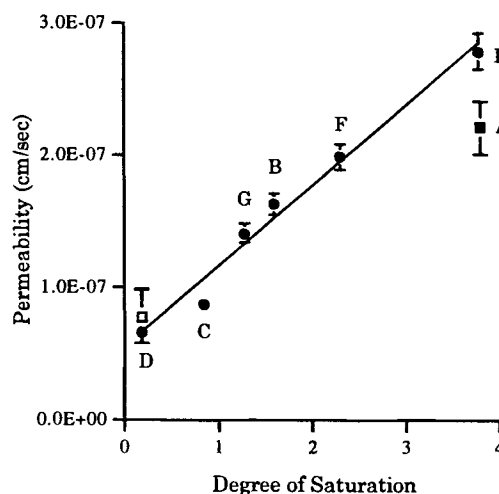


Fig. 6. Permeability coefficient of fluocinonide from topical solutions across silicone membrane as a function of the degree of saturation: solution A (filled square), solution B through G (filled circles) and Topsym™ solution (open square).

with drug concentration. The activity coefficient reflects the propensity of the drug to permeate through the membrane. It can, therefore, be inferred that using supersaturated drug vehicles, this propensity increases with the concentration and that drug flux through the silicone membrane can be raised to an extent beyond what would be anticipated based on ideal donor concentration and partition coefficient considerations only. This means that, when the same vehicle is used, drug flux can be increased supra-proportionally with increasing donor drug concentration. This observation is supported by significantly different permeabilities ($p = 0.01$, Student *t*-test, non-paired) obtained for formulations B and G (see Fig. 6), which have exactly the same vehicle composition.

In Vivo Human Skin Blanching Assay

Two clinical trials were carried out to estimate *in vivo* availability of fluocinonide from topical solutions with different degrees of saturation. Each series (see Table 1) was investigated in a different group of 12 volunteers. In series 1, the visually obtained AUBC values were 707, 837, 813, and 841 for solution A, B, C, and D, respectively, and 1142 for Topsy™ solution and are depicted in Fig. 7. Formulation A showed a slightly, but significantly lower AUBC than formulations B, C, and D with comparable AUBC values, which might have been caused by crystallization of drug with a concomitant decrease in concentration. Topsy™ solution, with its doublefold concentration compared to formulations A-D showed a significantly higher AUBC. No increase of pharmacodynamic response was observed when the degree of saturation was augmented within a formulation system at the same concentration level.

In series 2, the visually assessed AUBC values were 246, 333, 373, 473, and 1003 for formulations E, F, G, C, and Topsy™ solution, respectively. The AUBC values increase with increasing concentrations, and are inversely related to the degree of saturation. Significant differences were detected

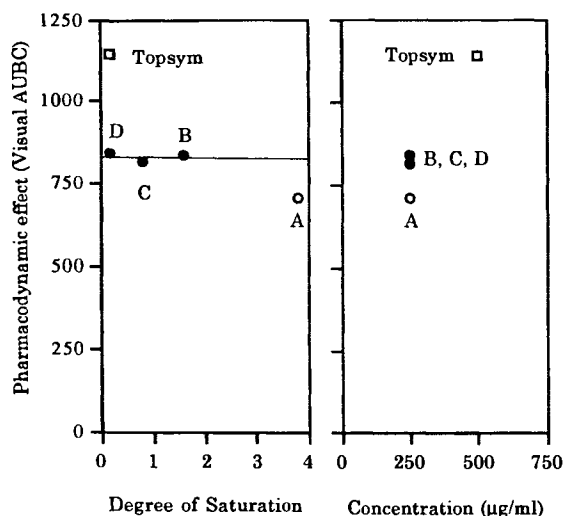


Fig. 7. Pharmacodynamic effect (area under the visually assessed blanching curve) for the fluocinonide topical solutions of series 1. Solutions A to D (circles) had the same concentration-level of 250 µg/ml; whereas solution A is outlined (open circle) for crystallisation might have occurred leading to a slightly weaker pharmacodynamic effect. Topsy™ solution (open square) had a concentration of 500 µg/ml.

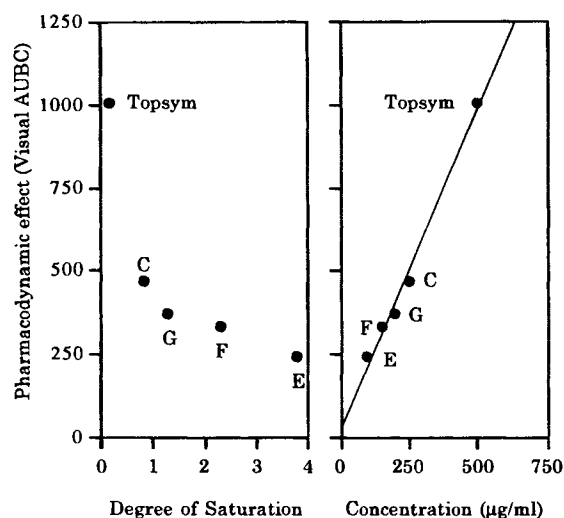


Fig. 8. Pharmacodynamic effect (area under the visually assessed blanching curve) for the fluocinonide topical solutions of series 2. The degree of saturation is increasing with decreasing concentration levels.

between E and G, E and C, F and C, and G and C. Figure 8 depicts the AUBC values in relation to concentrations and degrees of saturation. In this series, the pharmacodynamic responses were linearly related to the concentrations of the drug in the vehicles, irrespective to the degrees of saturation. Note that due to the different volunteer pools used in the two clinical trials, different AUBC values were gained for the two formulations (solution C and Topsy™) which were tested in both trials. The results gained by visual blanching response assessment were paralleled by those obtained from a chromameter (CR-300, Minolta Ltd., Dietikon, Switzerland; data not shown).

CONCLUSIONS

In the present work, supersaturated fluocinonide solutions containing polyvinylpyrrolidone K 25 as an antinucleant polymer were produced. In contrast to previous publications, the stability of supersaturated solutions was investigated under worst case conditions (nucleation-enhancing surface, intense stirring), since it has been suggested, that the rough surface, the particles and chemical compounds present on human skin would promote crystallization of supersaturated systems.

The results from the *in vitro* permeation studies using synthetic membranes reflect the findings recently published in the literature (7,8), since permeabilities were in correlation with the degree of saturation. The pharmacodynamic responses observed in the *in vivo* human skin blanching assay were related to the respective concentrations, and were found to be independent of the degree of saturation. Superior blanching responses were observed following application of Topsy™ solution, which proved that the end-point of maximal pharmacodynamic response was not yet attained with the concentrations present in the different fluocinonide formulations. Accompanying experiments showed that blanching responses were unaffected by the method of occlusion as comparable results were achieved by occluded application under Blenderm™ tape and with the use of aluminum chambers (Finn™ Chambers, Epitest Ltd., Finland). Furthermore, the application of formulation E (lowest

concentration, and highest drug escaping tendency) at 100%, 50% and 25% doses in terms of the amount of formulation per unit area demonstrated no loss of blanching response, which is an indication that no significant depletion of the drug formulation occurred during the 6 hour application period on skin. The fact, that the blanching responses were concentration related and independent of the degree of saturation is in opposition to the findings of this and other *in vitro* investigations performed with synthetic membranes (7,8).

An early publication of Haleblan and co-workers (21) reported, pharmacodynamic blanching responses of fluocinonide in propylene glycol/water gel bases were hardly affected by variation of the propylene glycol concentration in the range of 0 to 40% nor by the application manner (open or occluded). Additionally, the *in vitro* penetration through excised human skin was not markedly influenced by propylene glycol concentration variations in the range between 5 and 40%. Another publication by Møllgaard and Hoelgaard (22) demonstrated that the *in vitro* human skin permeation rate of estradiol is not affected by different amounts of propylene glycol present in the formulation (above a certain threshold level). In our experiments, the concentration of propylene glycol was between 10% and 40%, which was considered not to affect the skin penetration to a great extent.

Furthermore, Møllgaard and Hoelgaard demonstrated there was no relationship between degree of saturation of estradiol solutions and the permeation across excised human skin *in vitro*. They presumed the solute activity in the formulation only to be secondary, while the drug permeability is mainly influenced by a vehicle effect on the barrier function.

From the literature data and our findings we conclude that supersaturation enhances *in vivo* skin penetration of fluocinonide only by the factor of which the magnitude of concentration in the respective formulation was increased. Additionally, the presence of propylene glycol (penetration enhancer), and other factors inherent to the drug and formulation, may already contribute to an optimised skin penetration and the contribution of supersaturation to the overall skin penetration is considered to be minimal.

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